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L6: Entry 1 of 1 File: USPT Jun 3, 1997

DOCUMENT-IDENTIFIER: US 5635370 A
TITLE: DNA encoding BEHAB, a brain hyaluronan-binding protein, and recombinant expression systems for production of BEHAB polypeptides

DEPR:
To isolate rat cDNA clones encoding HA-binding proteins involved in neural development, an unamplified postnatal day 12 rat brain .lambda.gt10 cDNA library is screened with rat aggrecan clone pRCP 4 encoding the HA-binding region (described by Doege, K., et al., J. Biol. Chem. 262:17757-17767 (1987)). A total of 3.2.times.10.sup.5 recombinants are screened resulting in two positives. The library is rescreened with one of these clones, resulting in 15 additional clones. 4.times.10.sup.4 phage (per 150 mm plate) are plated with E. coli C600 bacteria, immobilized onto nitrocellulose filters, and prepared for hybridization using standard techniques. Filters are prewashed for 1 hour in 1M NaCl, 0.1% sodium dodecyl sulfate (SDS), 20 mM Tris-HCl (pH 8.0) and 1 mMEDTA at 65.degree. C. Filters are then prehybridized for an additional 4 to 6 hours in 50% formamide, 5.times.SCC (1.times.SCC=0.15M sodium chloride, 0.015M sodium citrate), 1% SDS, 1.times.Denhardt's (0.02% Ficoll, 0.02% bovine serum albumin (BSA, Fraction V), 0.02% polyvinylpyrrolidone), 50 mM sodium phosphate (pH 6.7), and 100 .mu.g/ml salmon sperm DNA at 37.degree. C. Hybridization is carried out in the identical solution with the inclusion of 10.sup.6 cpm pRCP 4 probe/ml for 24 hours at 37.degree. C. For all experiments, radiolabelled probes (.sup.32 P-dCTP, Amersham) are prepared by random priming (Boehringer Mannheim Corp., Indianapolis Ind.) gel purified cDNA inserts, followed by the removal of unincorporated radionucleotides (NICK column, Pharmacia). One post hybridization wash is in 2.times.SSC, 0.1% SDS and one in 0.2.times.SSC for 1 hour each are performed at room temperature. Phage DNA is isolated using DE52 (Whatman) and the cDNA insert excised by EcoRI digestion. The insert size of the clones are determined and partial restriction maps are prepared to eliminate redundant clones. The cDNA is gel purified (Gene-Clean.RTM., Bio 101), eight clones subcloned into pBluescript.RTM. KS+ (Stratagene, LaJolla, Calif.) and transformed into DH5.alpha. (GIBCO BRL, Gaithersburg, Md.).